Remarks

I. Status of the Claims

All of the claims originally filed with this application and previously entered have been cancelled and new claims 38-57 have been entered.

II. The Amendments

Newly entered claims 38-57 closely parallel claims that have been cancelled. The main difference is that original claim 11 (now claim 38) has been limited to certain specific amino acids. The new claims are fully supported by the specification and do not add new matter to the application, Their entry is therefore respectfully requested.

The Rejections

I. Rejection of Claims Under 35 USC § 103

On pages 3-11 of the Office Action, the Examiner rejects claims based upon the allegation that they are obvious in light of the combination of Volz (*Prot. Sci. 8*:24-28 (1999)); Enos-Berlage (*J. Bacteriol. 180*:6519-6528 (1998)), Verkhovskaya, *et al.*, (*Microbiol. 147*:3005-3013 (2001)) and Promega Technical Bulletin No. 117 (September 2002)). The references by Volz and Enos-Berlage were cited in previous Office Actions. The Verhovskaya reference teaches a method of "knocking out" genes by homologous recombination that, in part, uses a procedure described in the Promega reference in which bacteria are lysed, and cell debris is removed. The Examiner alleges that it would be obvious to apply this procedure to the *yjgF* gene and that the step of removing cell debris would amount to a purification of amino acids. The alleged motivation for combining references is that Volz and Enos-Verlag suggest the desirability of determining the function of *yjgF*.

Applicant respectfully traverses this rejection.

Comments Concerning Content of the Cited References

Applicant agrees with the Examiner that the Volz reference teaches that one approach to determining the function of the *E. coli yjgF* gene product is by analysis of its crystal structure. However, there are two important points that should be stressed. First, Volz is

concerned with analysis of the normal gene product, not mutated forms of the product. Second, the reference never suggests that a loss of *yjgF* gene function leads to increased bacterial amino acid production or otherwise suggests that bacteria with mutations in *yjgF* might be cultured and used to make amino acids.

Enos-Berlage is concerned with metabolic pathways by which Salmonella bacteria make thiamine. It was found that bacteria that have mutations resulting in a loss of both a primary and an alternative pathway can still make thiamine if they have a further mutation that results in a loss of a functional *yjgF* gene. This reference does not suggest that bacteria containing a mutated *yjgF* gene should be used for fermentatively producing amino acids and, as discussed further below, there are statements in the reference that actually appear to suggest that bacteria containing such mutations should *not* be used in the production of amino acids, *i.e.*, that teach away from the claimed invention.

Applicant does not dispute the assertion that Verhovskaya and the Promega Bulletin teach procedures for knocking out a gene and for purifying DNA from bacteria. The procedures described were not applied to the *yjgF* gene although, in principle, they could have been applied to *yjgF* or any other bacterial gene. However, it should be recognized that they would not *necessarily* be used. For example, one wanting to find the function of *yjgF* could use a different mutagenesis procedure or could try increasing, rather than decreasing, the activity of the gene and see what phenotypic consequences this had.

Although not actually used in rejecting claims, the Examiner also cites a paper by Kruse *et al.* (*Appl. Microbiol. Biotechnol. 59*:205-210 (2002)) in making arguments. This is cited as disclosing that "during routine growth of E. coli, culturing was performed in LB medium, while during the production of L-threonine using the same E-coli strain, culturing was in defined medium . . . . however, there is no evidence of record that LB medium is not conducive to amino acid production by E. coli." It is unclear why the Examiner believes that this reference supports the rejection of claims. It certainly does not suggest that all assay conditions are suitable for amino acid production. In fact, the switching from LB medium to a defined medium suggests otherwise. The Examiner also requests that Applicant provide evidence that amino acid production is altered by the conditions under which cells are

incubated. However, it should be appreciated that the initial burden during patent prosecution is for the Examiner to establish a prima facie case of obviousness. It should be apparent that Applicant does not believe that this has been done.

Isolation or Recovery of Amino Acids is Not Obvious from the References Cited

One of the main claim requirements that distinguishes Applicant's methods from the cited prior art is the isolation or the recovery[1] of amino acids from cultures of *yjgF* mutants. The Examiner seems to recognize that there are no express teachings in the references suggesting this step. However, he argues that lysing cells, centrifuging out cellular debris and collecting the supernatant, would be sufficient to meet the claim requirements. Since, according to the Examiner, this is disclosed in the prior art, the isolation or recovery of amino acids is inherent in the disclosure and Applicant's claimed methods are therefore obvious.

In response, Applicant submits that there are both factual and legal problems with this argument.

1. Factual Considerations

Applicant can see no place in the cited references where cells having a *yjgF* mutation were cultured, lysed, the lysate centrifuged and the resulting supernatant collected. The Volz reference is concerned with determining the crystal structure of the normal, unmutated *yjgF* gene product. There would be no need to culture bacteria with mutated forms of the gene to accomplish this. In fact, the use of mutant bacteria would be incompatible with the objective set forth in the paper. *i.e.*, analyzing the crystal structure of mutated, nonfunctional forms of the *yjgF* gene product would clearly not be a reasonable way for determining what the normal, functional, gene product does.

The Enos-Berlage reference is concerned with a problem of intermediary metabolism, *i.e.*, determining what mutations allow bacteria to make thiamine when normal pathways of synthesis have been lost due to other mutations (see abstract and first three paragraphs of the

[1] Claim 38 refers solely to the isolation of amino acids whereas claim 48 allows for either isolation or recovery. Although, for convenience, the arguments presented simply refer to "recovery," it will be understood that claim 48 actually requires both recovery and also a determination of the amount of amino acid recovered. Thus, merely collecting fermentation broth would not meet the requirements of claim 48, the collected material would need to also be assayed for amino acid levels.

reference). PCR amplification and sequence analysis allowed the authors to conclude that mutations in the *yjgF* gene could confer this capability (see page 6520 of the reference, second column under the heading "Localization of *yjgF* mutations by PCR amplification"). However, the reference does not suggest a relationship between mutations in *yjgF* and amino acid production or that mutant cells be lysed, cellular debris removed and the supernatant kept. Collecting supernatant as such would certainly not be inherent in carrying out PCR amplifications or sequence analysis. Applicant believes that procedures of this type normally involve the extraction of nucleic acids from cells and the elimination, not isolation or recovery, of amino acids.

The references by Verhovskaya and the Promega Bulletin, like the Enos-Berlage reference, are concerned with the purification of DNA, not amino acids. These references appear to have been cited to indicate that there is a step in preparing DNA in which cellular debris is removed from a cell lysate and to suggest that this amounts to a purification of amino acids. However, as far as Applicant can tell, there is no reason to conclude that the procedure used in these references is the same as the one used by Enos-Verlage. Thus, their relevance to the present rejection is unclear.

Beyond this, there appears to be a difference in what the Examiner and Applicant understand the term "isolation" or "purification" to mean. The Examiner seems to take the position that if amino acids are transiently enriched in any step in a process, this constitutes a purification even if they are discarded in the next step. However, Applicant submits that, in ordinary usage, one looks at the end result of a process to determine if purification or isolation has occurred. The processes of Enos-Verlag, Verhovskaya and the Promega Bulletin are all expressly designed for the isolation of DNA. Their end result is not the purification of amino acids, but their elimination along with all other cellular components that contaminate the DNA. Using the terms "isolation" and "purification" in accordance with their ordinary meaning, one cannot reasonably interpret a procedure that has as its end result the elimination of amino acids as a procedure for purifying amino acids.

2. Legal Considerations

As discussed further below, there is no basis in patent law for combining references and then concluding that the process is not novel, in part, because some elements are inherently present. In the present case, the only valid issue is one of obviousness. More specifically, the issue is whether a combination of references that never suggest a relationship between a mutation in the *yjgF* gene and amino acid production and which admittedly do not actually or inherently disclose the claimed invention (*i.e.*, the references are not anticipatory) renders claims to a process for producing amino acids which require that they be isolated, or recovered and quantitated, obvious. Applicant does not see any way that this could occur. From an obviousness perspective, how can references make the use of *yjgF* mutants for the fermentative production of amino acids in bacteria obvious when they do not teach any relationship between mutation and increased amino acid production?

The Examiner Mixes Novelty and Obviousness in a Legally Impermissible Way

None of the references cited disclose or in any way suggest that there is a relationship between mutations in the *yjgF* gene and amino acid production. Therefore, even if the references were combined and all of their teachings were read by one of skill in the art, the claimed invention would still not be disclosed. Apparently recognizing this, the Examiner argues that when the references are combined, the increase in amino acid production in cells carrying mutations would inherently be present in the resulting process and therefore teachings leading to the conscious recognition of the claimed invention are not needed.

This argument mixes novelty and obviousness elements in a way that is legally impermissible. A *novelty* rejection requires that all of the elements of a claim be expressly or inherently present *within the four corners of a single reference.* Here the Examiner has made what amounts to a novelty rejection by combining references. An *obviousness* rejection requires that a reference or combination of references make a claimed invention obvious. Although patent law recognizes inherent novelty, there is no such thing as inherent obviousness, *i.e.*, in order to be obvious, a reference or combination of references must make one of skill in the art consciously aware of the invention claimed. The combination of references cited clearly fails to do this.

Enos-Berlage Teaches Away From the Use of yjgF Mutants for Amino Acid Synthesis for the Salmonella Mutants Studied

The Enos-Berlage reference is concerned with strains of Salmonella bacteria that have undergone mutations to eliminate metabolic pathways that are normally present and that, as a result, must be grown under special conditions. There is no indication that the conditions described would be conducive to amino acid production. In addition, the reference has statements that suggest that a loss of *yjgF* function due to mutation should decrease, not increase, amino acid production. For example, on page 6526, first column, second full paragraph under the "Discussion" section, the reference states:

> We suggest that the yjgF mutation results in the partial block of at least one step in isoleucine biosynthesis (by an as yet undefined mechanism) subsequent to the reaction catalyzed by threonine deaminase . . .

Thus, *yjgF* mutations are suggested to block, not promote, bacterial amino acid production. Applicant submits that the reference therefore teaches away form the methods claimed.

Conclusion

In light of the discussion above, Applicant believes that all of the Examiner's rejections have been overcome. It is therefore respectfully requested that these rejections be withdrawn and that the claims now pending in the application be allowed. Early notice to this effect is earnestly solicited. If, in the opinion of the Examiner, a phone may expedite the prosecution of this application, the Examiner is invited to call Applicant's undersigned attorney at (240) 683-6165.

Respectfully submitted,
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